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TRAINING MANUAL FOR TERATOLOGY

K. D. Courtney, et al

**National Environmental Research Center
Research Triangle Park, North Carolina**

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TRAINING MANUAL FOR TERATOLOGY

by

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ABSTRACT

This report is an introduction to the basic techniques involved in teratology testing. General topics covered include the handling of animals, compound preparation and administration, dosage calculations, and coding techniques. Teratology techniques include fetal fixation, alizarin staining, and the examination of fixed and stained fetuses for soft tissue and skeletal anomalies. Suitable protocol sheets covering data obtained before sacrifice, after sacrifice, and from subsequent fetal examinations are included. A glossary of the most common malformations and their description is present. The outlines of a teratology experiment with Vitamin A is included as a suggested training exercise. This experiment and the techniques described in the manual may be used to train laboratory personnel in the techniques and designs of teratology experiments.

TRAINING MANUAL FOR TERATOLOGY

INTRODUCTION

Teratology is the study of birth defects. It is derived from the Greek word "teratos," which means monster, and indeed in ancient times children with birth defects were considered monsters and evil omens. It is now known that birth defects can occur any time there is interference with, or malfunctioning of, the normal processes of development. The causes of birth defects are as varied as the types of defects themselves. Genetic factors, maternal disease, too much or too little of vital nutrients, radiation, oxygen deprivation, or the presence of foreign chemicals may all contribute to the formation of birth defects. The defects can be external such as shortened limbs or cleft lip, or internal such as heart or kidney malformations. Cellular defects may also occur and can make their presence known by malfunctioning organs or mental retardation.

From day to day, human beings come in contact with large numbers of diverse chemicals, including food additives, drugs, and other natural and synthetic compounds present in the environment. There is great need for the proper testing of these substances to determine their ability to cause birth defects since seemingly innocuous drugs (e.g., thalidomide) and environmental contaminants (e.g., methylmercury) are known to have caused serious malformations.

BASIC TECHNIQUES

EXPERIMENTAL ANIMALS

Strains of Mice

Mice are classified according to strains. An inbred strain is homogeneous for a number of specific genes while a random-bred strain is genetically heterogeneous. The studies described here utilize the random-bred, all purpose, white laboratory mouse.

Handling of Mice

The description below is for right-handed people; left-handed people should reverse the procedure.

To handle mice properly, the worker should always be firm but gentle and never frighten the animals. A mouse may be picked up by the tail but should not be suspended in this position for too long. For transportation across short distances (e.g., from cage to scale) it is best to place the animal on your left forearm while maintaining your hold on the tail with your right hand.

For administration of a compound to the mouse by any route, a firm grasp is needed to prevent the animal from either escaping or nipping. This is achieved by holding the mouse by the tail and placing it on a cage top or other rough surface that it can grab. One then attempts to have the mouse extend its body by either allowing it to pull away from one while holding the tail or by gently pulling the mouse backwards after it has grabbed the cage top. Once the mouse has extended its body, all the skin at the nape of the neck should be firmly grasped with the thumb and index finger of the left hand so that the animal's forelimbs are immobilized. Care must be taken not to choke the mouse. The tail should then be placed between the ring finger and little finger of your left hand. The right hand is now free to handle a syringe.

Techniques of Compound Administration

Compounds are administered by oral intubation or by subcutaneous injections. For oral intubation, a feeding needle is used on the syringe. An 18-gauge needle with a blunt end or a needle with an 18-gauge ball tip works well. These needles should be approximately 2 inches long. The intubation needle is put into the most posterior portion of the oral cavity, causing the mouse to swallow. When the mouse swallows, the needle is guided down the esophagus and into the stomach. The proper volume of vehicle, the substance in which the compound is dissolved or suspended to facilitate administration, is expelled from the syringe, and the needle gently withdrawn.

When the compound is to be injected subcutaneously, a hypodermic needle is used on the syringe. A needle of 25 gauge and 1 inch length works well. The mouse is grasped and held in the same manner described above. The needle is inserted into the skin at the nape of the neck, which is held between the thumb and index finger. The needle should always enter the skin at an angle of 45 degrees or less. It should be inserted at least 0.5 inch to minimize leaking upon withdrawal. The needle should be withdrawn at precisely the same angle to prevent tearing of the skin.

Safety Procedures

A strong point must be made concerning safety procedures. It must be remembered that many compounds are being studied for their teratogenic potential because so little is known about them. Thus, at all times, rubber gloves are worn when one is working with test compounds or animals. Volatile compounds or solvents are to be used in the hood. It may even be necessary to administer a volatile compound in a hood. All pipetting is done by mechanical devices, not by mouth. All intubation needles are to be rinsed with acetone after they are washed. This step is important since most of the compounds are not soluble in water. The washing procedure will mechanically remove any suspensions, and a rinse with acetone will ensure that any residual will be washed away and the needle will be dry. Because hypodermic needles will be disposable, they will not require this attention.

Caging

Pregnant mice are caged at a density of no more than six and no less than two mice per cage, with each cage numbered sequentially. The mice within the cage are identified individually by coding 1 through 6, applied with an ear punch. The mouse number is the cage number followed by the ear punch code number; e.g., the mouse in cage 12 with the ear code 5 is mouse number 12-5.

Coding by Ear Punch

Individual mice within a single cage are identified by ear coding. To ear code, one should first hold the mouse firmly in the left hand as described for compound administration. With the ear punch in the right hand, a swift, sure movement will produce a small hole in the ear. The placement of the hole will determine the mouse ear code number, as depicted in the scheme in Figure 1.

Condition of Colony

It must be remembered at all times that your experiment is only as good as the animals you use. Clean cages are necessary to minimize outbreaks of diseases or illness that can spread throughout the whole colony. Also, food and water should be available for the mice at all times. If an animal has been without food, water, or both, it should be immediately noted in the records. This notation is extremely important since it has been shown that mice deprived of food produce malformed fetuses.

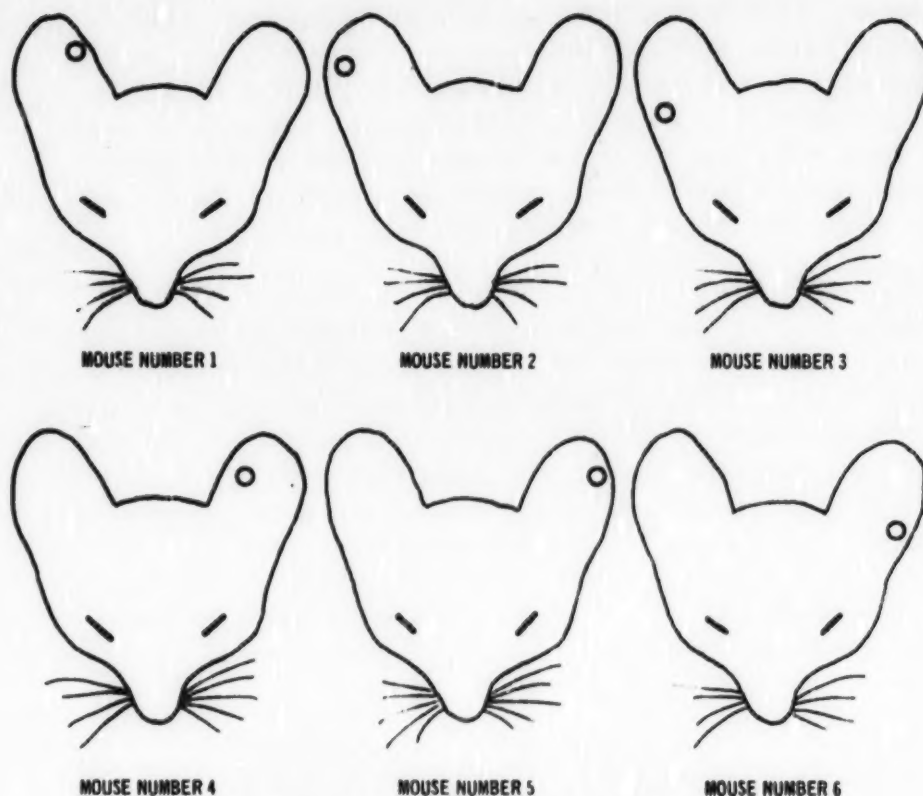


Figure 1. Scheme for determining mouse ear code number.

COMPOUND PREPARATION

Upon arrival in the laboratory, all compounds are logged by sequential number in a book indicating the date of arrival, source, batch number, purity, storage, and any other pertinent information. This log number is marked on the container of the compound.

Solutions

Either solutions or suspensions may be used for oral administration; only solutions are used for subcutaneous administration. When a solution is used, all of the compound must be dissolved, and the solution well mixed. If the compound is stable in the solution, one solution may be made for the entire experiment. If there is any chance of decomposition of the compound in the solution, then a new solution must be made daily. A solution should always be stored in the dark to prevent photodecomposition. Storage in the cold is also recommended. If a solution is stored in the refrigerator or freezer, it should be allowed to warm up to room temperature before being administered to an animal. Extreme caution should be used if heat is applied to these solutions since they could decompose.

When a solution is to be injected, the pH should be within the biological range of pH 6.0 to 8.0 in order to prevent discomfort and stress to the mouse, as well as lesions and fibrotic areas. Solvents that may be injected are water, saline, buffers, and dimethylsulfoxide (DMSO). Corn oil, organic solvents, and suspending media are not compatible with tissue fluids and, therefore, should be intubated into the stomach.

Suspensions

In order to make a suspension that will stay suspended long enough to enable the syringe to be filled and the mice to be treated, one probably needs to grind the compound to a small particulate size. The smaller the size of the particle, the longer it stays in suspension. An ordinary mortar and pestle is adequate for most compounds. Occasionally a motor-driven pestle is necessary. The suspending agent may be water or saline, but usually a more viscous substance, such as corn oil, honey, sugar solutions, or carboxymethylcellulose, is used.

Suspensions must be made daily. This can easily be achieved by weighing the daily supply of compound in as many vials as are needed for the experiment. The vials are then stoppered, labeled, and stored appropriately. Each morning, sufficient suspending agent is added to a vial, and the suspension thoroughly mixed. It is necessary to make daily suspensions in order to minimize the error in dosage created by improper sampling of suspensions.

When suspensions are used, it is recommended that the syringe be filled directly from the vial and then the needle be placed on the syringe. In this way, it is easier to remove air bubbles from the syringe.

Color Coding of Solutions and Mice

Each solution or suspension is labeled with colored tape. The cage containing the mice that receive that solution or suspension is also labeled with the same colored tape, thus minimizing the need for written labels as well as minimizing errors.

Dosage Calculations

The dose is always expressed in terms of kilograms (kg) of mouse per day. The average body weight of the mice in a cage on gestational day 6 is used for the calculations. Thus, on day 6 of gestation, the mice in cage 12 are weighed. The numerical average of these weights is calculated as shown in the following example:

<u>Mouse no.</u>	<u>Weight, grams</u>
12-1	28.1
12-2	22.2
12-3	24.6
12-4	23.7
12-5	27.2
12-6	25.4
	151.2 = total

$$\frac{151.2}{6} = 25.2 \text{ grams} = \text{average mouse weight in cage 12}$$

The mice in cage 12 have been assigned compound (cmpd) Z at a dose of 150 milligrams (mg)/kg/day. To make the appropriate solution for the mice in cage 12, the following calculations are used:

$$\frac{150 \text{ mg of cmpd Z}}{1000 \text{ g of mouse}} = \frac{X \text{ mg of cmpd Z}}{25.2 \text{ g of mouse}}$$

This equation should be read as follows: if 150 mg of compound Z are required for 1000 g of mouse (= 1 kilogram), then how many mg of the compound (X) are required for 25.2 g of mouse. This equation is solved by cross multiplying as follows:

$$\frac{150 \text{ mg cmpd Z}}{1000 \text{ g mouse}} \times \frac{X \text{ mg cmpd Z}}{25.2 \text{ g of mouse}}$$

$$(1000 \text{ g mouse}) (X \text{ mg cmpd Z}) = (150 \text{ mg cmpd Z}) (25.2 \text{ g mouse})$$

$$1000 X = 150 \times 25.2$$

$$1000 X = 3780.0$$

$$X = \frac{3780.0}{1000}$$

$$X = 3.78 \text{ mg of compound Z}$$

Thus each mouse in that cage receives 3.78 mg of compound Z every day.

Assuming that compound Z is soluble and stable in the vehicle, then a solution is made as follows:

1. Each mouse receives an injection of 0.1 milliliter (ml) daily for 10 days. Thus each mouse needs 10 x 0.1 ml or 1.0 ml of solution for the experiment.
2. Since there are six mice in the cage, the quantity of solution needed for the experiment is six times 1.0 ml of solution or 6.0 ml.
3. As to the total volume required, allowances must be made for the amount that is needed to fill the syringe properly. Thus, to have adequate solution for an individual cage of mice for the whole experiment, a total volume of 10.0 ml is more than enough.

Calculations for Making a Solution

The quantity of compound Z in a volume of 0.1 ml needed for each mouse for each day is 3.78 mg. To make 10.0 ml of solution, the problem is approached in the same manner as the following:

$$\frac{3.78 \text{ mg cmpd Z}}{0.1 \text{ ml solvent}} = \frac{X \text{ mg cmpd Z}}{10.0 \text{ ml solvent}}$$

To solve this equation, first cross multiply as follows:

$$\begin{array}{ccc} 3.78 \text{ mg compd Z} & \searrow & X \text{ mg compd Z} \\ 0.1 \text{ ml solvent} & \nearrow & 10.0 \text{ ml solvent} \end{array}$$

$$(0.1 \text{ ml solvent}) (X \text{ mg compd Z}) = (3.78 \text{ mg compd Z}) (10.0 \text{ ml solvent})$$

$$(0.1) (X) = (3.78) (10.0)$$

$$0.1 X = 37.8$$

$$X = \frac{37.8}{0.1}$$

$$X = 378 \text{ mg of compound Z}$$

Thus, 378 mg of compound Z should be weighed out and dissolved in 10.0 ml of solvent. Each injection will use 0.1 ml of the solution.

Practice Problems

In the following problems, determine the solution or suspension that would be required.

1. Compound D will be administered at a dose of 84 mg/kg as a stable solution. The mice in the cage weigh: 22.4, 23.6, 25.8, 26.1, 24.7, and 23.2 g.
2. Compound E will be administered at a dose of 52 mg/kg as a suspension. The mice in the cage weigh: 23.8, 24.3, 24.6, 25.2, and 25.9 g.
3. Compound F will be administered at a dose of 115 mg/kg as a nonstable solution. The mice weigh: 24.2, 24.7, 25.8, 26.1, 22.0, and 23.3 g.

FETAL FIXATION AND STAINING

Fetal Fixation

Fetuses should be hardened to ensure proper necropsy. Bouin's solution is a preservative and fixative which accomplishes this. It consists of a saturated solution of picric acid mixed with formaldehyde and glacial acetic acid. The solution will harden a mouse fetus in about 3 days. Fetuses left in this solution for more than 3 weeks become too brittle for necropsy.

Modified Bouin's Solution:

1. Prepare picric acid: 30 g dissolved in 1900 ml H₂O.
2. Add 1900 ml of formaldehyde.
3. Add 190 ml of glacial acetic acid.
4. Mix well.

Alizarin Staining

Alizarin red S is used to stain the skeleton, permitting examination for skeletal defects. Fetuses from an entire litter are placed in a jar with 70 percent propyl or ethyl alcohol for at least 24 hours. They may be stored indefinitely in 70 per-

cent propyl or ethyl alcohol. The amount of alcohol should be approximately 10 times the volume of the fetuses. The fetuses are put into the alcohol to fix or preserve the tissues.

When the fetuses are sufficiently fixed by the alcohol, usually in 1 to 3 days, all internal organs are removed with small forceps. The heart and lungs are removed through an opening made in the diaphragm, leaving the rib cage intact. Also, a fat pad located on the back of the neck and between the shoulder blades is carefully removed with scissors and forceps.

After the internal organs have been removed, the fetuses are placed in a 1 percent potassium hydroxide (KOH) solution until the bones are visible through the skin. This step may take from 1 to 7 days. The KOH solution is then poured off and replaced with the following solution:

distilled H ₂ O	800 ml
1% KOH solution	35 ml
glycerin	200 ml
alizarin solution	10-15 drops

Solutions are as follows:

1% KOH solution	- 1 g of potassium hydroxide in 100 ml of distilled water.
50% glycerin	- equal amounts of glycerin and distilled water - mix well.
alizarin solution	- 500 mg alizarin red S in 100 ml of distilled water.

Within 1 to 2 days the fetal bones should be stained red. When the desired depth of coloring of the bones has occurred, the staining solution is poured off; the fetuses are rinsed with water and placed in a 50 percent glycerin solution. After 1 or more days in the glycerin solution, the fetuses can be examined for skeletal defects. Fetuses that are kept for long indefinite periods should be preserved in 100 percent glycerin, to which some crystals of thymol have been added to prevent fungal growth.

EXPERIMENT DESIGN

MATERNAL DATA

Experiment Schedule

The schedule shown in Table 1 was designed for use with CD-1 mice; with some compounds or with other strains, variations of this design could be necessary. The mice may be bred by a commercial supplier and delivered to the teratology laboratory before they are 5 days pregnant, or bred at the laboratory.

The mice are mated according to the following plan. One group of mice are mated on Friday night with detection of a vaginal plug on Saturday morning. From this breeding, there should be 100 pregnant mice, with the first day of gestation being on Saturday as outlined in Table 1. Then, on the following Thursday (day 6 of gestation), these mice are weighed and coded by ear punch. The compound they are to receive is prepared for the entire experiment. The compound is administered daily for the next 10 days, starting on Friday (day 7 of gestation). On gestational day 17, Monday, these animals are weighed and sacrificed. The fetuses are removed, weighed, and stored in Bouin's solution until necropsied. Some litters are stored directly in 70 percent propyl alcohol for staining the skeleton with alizarin red S. (See earlier

Table 1. SCHEDULE SHOWN FOR 4 WEEKS FOR GROUPS OF MICE A, B, C, D, E, F
This schedule will continue as such.

<u>Sun</u>	<u>Mon</u>	<u>Tues</u>	<u>Wed</u>	<u>Thurs</u>	<u>Fri</u>	<u>Sat</u>
		mate A	day 1 A		mate B	day 1 B
<u>Sun</u>	<u>Mon</u>	<u>Tues</u>	<u>Wed</u>	<u>Thurs</u>	<u>Fri</u>	<u>Sat</u>
	weigh A	treat A mate C	treat A day 1 C	treat A weigh B	treat A treat B mate D	treat A treat B day 1 D
<u>Sun</u>	<u>Mon</u>	<u>Tues</u>	<u>Wed</u>	<u>Thurs</u>	<u>Fri</u>	<u>Sat</u>
treat A treat B	treat A treat B weigh C	treat A treat B treat C mate F	treat A treat B treat C day 1 E	treat A treat B treat C weigh D	kill A treat B treat C treat D mate F	treat B treat C treat D day 1 F
<u>Sun</u>	<u>Mon</u>	<u>Tues</u>	<u>Wed</u>	<u>Thurs</u>	<u>Fri</u>	<u>Sat</u>
treat B treat C treat D	kill B treat C treat D weigh E	treat C treat D treat E mate G	treat C treat D treat E day 1 G	treat C treat D treat E weigh F	kill C treat D treat E treat F mate H	treat D treat E treat F day 1 H

section entitled Fetal Fixation and Staining for preparation of Bouin's solution and alizarin staining technique.) Other strains of mice may be sacrificed on gestational day 18 or 19 since they have longer gestational periods. The purpose is to sacrifice the mice 1 or 2 days before delivery to recover the fetuses.

Another group of mice are mated on Tuesday so that there will be 150 pregnant mice with a day 1 of gestation on Wednesday. Then on the following Monday (day 6 of gestation) these mice are weighed, and ear coded; the compound is then prepared for this group. On Tuesday (day 7 of gestation), the treatment begins and continues for 10 days. On Friday (day 17 of gestation), the mice are sacrificed as indicated above.

Protocol Sheet A - Before Sacrifice

Protocol sheet A, a sample of which is provided on the next page as Figure 2, is filled out when the pregnant mice arrive in the teratology laboratory. The sheet should be completed as follows:

The cage number is placed in the upper right-hand corner. The cages are numbered sequentially. Assignment of the compound and the doses is done on a random basis; however, all the mice in any single cage receive the same treatment.

The date of day 1 means the date (month/day) of the first day of gestation, which is defined as the date when a vaginal plug was found in the female.

The compound and its log number are entered.

The vehicle is defined as the substance in which the compound is dissolved or suspended to facilitate administration. There are always animals that receive only the vehicle; they serve as controls for those receiving the compound. For these control animals, the vehicle is entered and the compound listed as NONE.

The route of administration (injection, intubation) is listed as well as the volume administered in a single treatment.

The dose is entered in terms of quantity of compound per kg of mouse per day.

Both the dates of treatment (e.g., March 12-21) and the days of gestation (i.e., days 7-16) will be listed.

As above, the date the mouse is sacrificed and the day of gestation are entered by date and day sacrificed. The days of treatment and sacrifice could vary with the compounds tested and the strain of mouse utilized.

On gestational day 6, the animals are weighed and coded. This weight is placed in the column under the appropriate mouse number across from day 6 weight.

During the course of treatment, the dates of any maternal deaths are entered in the appropriate boxes across from died (date).

Cage No. _____

Mouse strain _____

Date of day 1 _____

Compound _____ Log no. _____

Vehicle _____ Dose _____ /kg/day

Route _____ Volume _____ ml/day

Dates and days treated _____

Date and day sacrificed _____

Mouse ear no.	1	2	3	4	5	6
Day 6 weight						
Day sacrifice weight						
Liver weight						
Gravid uterus weight						
Total live litter weight						
No. live fetuses						
No. dead and resorbed fetuses						
Non-pregnant						
Died (date)						

Figure 2. Protocol sheet A.

Protocol Sheet A - Day of Sacrifice

The day the animals are sacrificed, they are weighed and this weight is entered on the sheet under day sacrifice weight.

After sacrifice, the animals are placed on their backs on plastic-backed absorbant paper. The fur on the abdomen is thoroughly wetted with a dilute solution of detergent from a squeeze bottle, preventing the fur from flying when the abdomen is opened. With the use of a pair of dissecting scissors, the abdomen of the animal is opened by cutting through the skin and muscle from the region of the bladder to the thorax. This incision exposes the uterus, liver, and other maternal organs. The maternal liver and the gravid uterus are removed, each is weighed independently, and the weights are entered under liver weight and gravid uterus weight. The gravid uterus is the entire uterine mass (both horns), with fetuses (live and dead) and amniotic fluid contained within.

After the weighing, the gravid uterus is carefully opened and the fetuses removed. Care should be exercised not to damage the fetuses. The live fetuses from a single litter are counted and entered as no. live fetuses. They are then weighed, and this value is entered under total live litter weight.

After the weighing, the litter destined to be necropsied is placed in a single compartment of a plastic tray that contains Bouin's solution. For large fetuses, a transverse abdominal cut may be necessary to permit the Bouin's solution to penetrate the internal organs fast enough to prevent rotting. The cage number and specific mouse number should be noted, with wax pencil, on the lid of the compartment on a piece of tape. Thus, the litter from mouse number 5 in cage 12 would have a piece of tape reading 12-5 above its compartment. The cage number always is first, followed by the mouse number.

A litter destined for alizarin red S staining is counted, weighed, and then placed in a jar containing 70 percent propyl alcohol. A transverse abdominal cut may be made in the fetuses to enhance fixation. The jar, not the cap, should be labeled with the mouse number.

In addition to live fetuses, a gravid uterus may contain dead fetuses or resorption sites. A dead fetus generally appears as a well formed, pale fetus with no response to stimulation and no apparent umbilical circulation. A resorption site appears as a macerated fetus or a small dot of black tissue. This is the result of fetal death and subsequent autolysis (dissolving) and resorption. At the time of examination of the uterus, the dead fetuses may vary greatly in size and form, depending upon the length of time they have been autolyzing. They may appear as pale fetal forms or very small masses termed resorption sites.

When found, dead fetuses and/or resorption sites are counted, and the number is entered under no. dead and resorbed fetuses.

Uteruses that do not appear to contain fetuses are carefully examined for the presence of small resorption sites. If none are found after careful examination, the animal is considered non-pregnant and should be so indicated by placing an "X" in the appropriate box.

FETAL DATA

Examination of Fetuses by Necropsy

Fetuses stored in Bouin's solution may be necropsied when they have become firm. Generally 3 days is sufficient for this to happen. Before necropsy, the fetuses are rinsed in water to remove excess Bouin's solution since it is an eye, nose, and throat irritant. Gloves are worn at all times during necropsy to avoid contact with both the test chemicals and the Bouin's solution.

The fetus is initially examined for external defects. Then the top of the head is cut off by inserting the scalpel into the mouth above and parallel to the tongue and slicing through the back of the skull. The palate and tongue are observed. Coronal sections are made through the nares, eyes, and cerebrum, and these portions are examined. The internal organs of the fetus are exposed by cutting, with a pair of scissors or scalpel, through the ventral body wall from the region of the bladder up through the thorax, and then across the abdomen at the level of the liver. By gently pulling the fore and hind limbs apart, the examiner inspects the heart and lungs. The liver and intestines should be examined and removed so that the sex of the fetus can be determined. This step is accomplished by the identification of either the testes or uterus. The testes are spherical bodies, one on each side of the bladder and against the posterior body wall. The uterus is a flattened "V"-shaped tube against the posterior body wall.

Each kidney is then sliced open so that the internal structure can be observed.

During the examination of a fetus, the size, shape, and location of organs or structures are carefully noted. Deviations from normal of any of these factors describe the majority of the malformations that will be observed.

Notations on specific anatomical malformations are found in the glossary at the end of this section.

Examination of Alizarin-Stained Fetuses

Alizarin-stained fetuses should remain in 50 percent glycerin during examination. At this stage of development, the bones of the cranium are almost completely ossified. The bones of the arms, legs, digits, ribs, and spinal cord are ossified while the carpals, tarsals, and sternebrae may not be.

The skeleton should be examined for the presence or absence of bones as well as the shape or length of the bones and degree of ossification.

Protocol Sheet B

Protocol sheet B, a sample of which may be found on the next page (Figure 3), is utilized at the time of fetal examination by necropsy or alizarin staining. It is designed to record descriptions of abnormal fetuses. The mouse number is placed on the upper right-hand corner. When the litter has been stained with alizarin red S, an "X" is placed after alizarin.

If all the fetuses in a litter are found upon examination to be normal, an "X" is placed after all normal.

In litters with both normal and abnormal fetuses, the normal fetuses are sexed and tallied. Each abnormal fetus is arbitrarily assigned a number, and its description occupies a single column on the protocol sheet.

There are seven columns on the protocol sheet that will accommodate seven abnormal fetuses. In a litter in which there are more than seven abnormal fetuses, two protocol sheets are used. This fact should be indicated by placing an "X" after two sheets used in the upper right hand corner.

The types of defects and their descriptions are found in the glossary. A single fetus may have more than one defect, e.g., clubfoot and cleft palate. There can also be more than one defect for each fetal structure. All defects are entered in the column across from the appropriate structure, by the use of the acceptable abbreviations as they appear in the glossary. For defects not listed in the glossary, a standard medical dictionary should be consulted.

All fetuses, both normal and abnormal, should be sexed, and the total number of males and females for the litter placed in the upper left corner under no. females, no. males. The sex of abnormal fetuses should also be entered as M or F in the line labeled sex/uterus/testes.

Glossary of Malformations

The malformations listed in the glossary are arranged in groups as they appear on protocol sheet B. Each malformation is followed by the appropriate abbreviation to be used on the sheet. Some malformations are not listed in the glossary since they occur infrequently. If any are observed, reference should be made to a textbook or medical dictionary and the full name of the malformation should be entered under the appropriate fetal column in one of the blank boxes.

Brain/Cranium

encephalocele - EN - protrusion of the brain through an opening of the skull.
The cerebrum is well formed and covered by transparent connective tissue.

exencephaly - EX - lack of skull with disorganized outward growth of the brain.

No. females _____ No. males _____ Mouse no. _____
 All normal _____ Alizarin _____ Two sheets used _____

Abnormal fetus no.							
Brain/cranium							
Right eye							
Left eye							
Jaw							
Palate							
Right front limb							
Left front limb							
Right front digits							
Left front digits							
Right hind limb							
Left hind limb							
Right hind digits							
Left hind digits							
Ribs							
Spinal cord							
Tail							
Intestines							
Heart							
Lung							
Sex/uterus/testes							
Right kidney							
Left kidney							

Figure 3. Protocol sheet B.

microcephaly - M - small head on normal sized body.

hydrocephaly - H - marked enlargement of the ventricles of the cerebrum.

craniorachischisis - CR - exposed brain and spinal cord.

Nose

enlarged naris - EN - enlarged nasal cavities.

single naris - SN - a single naris, usually median.

Eye

microphthalmia - M - small eye.

anophthalmia - A - lack of eye.

open eye - O - no apparent eyelid, eye is open.

Ear

anotia - A - absence of the external ear.

microtia - M - small ear.

Jaw

micrognathia - M - small lower jaw.

agnathia - A - absence of lower jaw.

aglossia - AG - lack of tongue.

astomia - AS - lack of mouth opening.

bifid tongue - BT - forked tongue.

cleft lip - CL - may be either unilateral or bilateral cleft
of the upper lip.

Palate

cleft palate - CP - a cleft or separation of the median portion of the palate.

Limbs

clubfoot - C - a foot that has grown in a twisted manner, resulting in an abnormal shape or position. It is possible to have a malposition of the whole limb.

micromelia - M - abnormal shortness of the limb.

hemimelia - H - absence of any of the long bones resulting in a shortened limb.

phocomelia - P - absence of all the long bones of a limb, the feet being attached directly to the body.

Digits

polydactyly - P - presence of extra digits. Since five is the normal number in the mouse, a polydactylous fetus would have six or more.

syndactyly - S - fusion of two or more digits.

oligodactyly - O - absence of one or more digits.

brachydactyly - B - smallness of one or more digits.

Ribs

wavy ribs - W - ribs may be any aberrant shape.

extra ribs - E - may have extra ribs on either side.

missing ribs - M - may be missing on either side.

fused ribs - F - may be fused anywhere along length of rib.

branched ribs - B - single base and branched.

Spinal Cord

spina bifida - B - nonfusion of spinal processes. Usually the covering ectoderm is missing, and the spinal cord is evident. Vertebrae may be missing, fused, or malshaped.

Tail

short tail - S - short tail, usually lack of vertebrae.

missing tail - A - absence of tail.

corkscrew - C - a corkscrew-shaped tail.

Intestines

umbilical hernia - UH - protrusion of the intestines into the umbilical cord.

ectopic intestines - EI - extrusion of the intestines outside the body wall.

Heart

dextrocardia - D - rotation of the heart axis to the right.

enlarged heart - E - either the atrium or the ventricle may be enlarged.

Lung

enlarged lung - L - all lobes are usually enlarged.

small lung - S - all lobes are usually small. Lobes may have immature appearance, e.g., look like a bunch of grapes.

Sex/uterus/testes

sex - M or F - the sex of every abnormal fetus should be entered.

undescended testes - U - testes are located anterior to the bladder instead of lateral. May be bilateral or unilateral.

agenesis of testes - AT - one or both testes may be missing.

agenesis of uterus - AU - one or both horns of the uterus may be missing.

Kidney

hydronephrosis - H - fluid-filled kidney, often grossly enlarged. This condition may be accompanied by hydroureter.

fused - F - kidneys fused, appearing as one misshapen kidney with two ureters.

agenesis - A - one or both kidneys missing.

misshapen - M - small, enlarged (normal internally), spherical, or odd-shaped kidneys.

SAMPLE EXPERIMENT: VITAMIN A

Vitamin A was selected as a model compound that can be used to produce many types of anomalies. Single large doses of vitamin A are teratogenic, and the day of gestation on which it is administered determines the types of anomalies produced. The following experiment with vitamin A has been designed to produce a large spectrum of types of anomalies.

EXPERIMENT DESIGN

Vitamin A palmitate is administered to CD-1 mice as a suspension in corn oil by oral intubation. The initial dose is 25,000 to 40,000 Units/mouse in 0.1-ml volume. Vitamin A is administered on a single day of gestation during the period of organ formation, days 8 - 13. All mice are sacrificed on day 17 of gestation.

On Friday, Saturday, Sunday, Monday, and Tuesday, mice are mated, resulting in the following groups:

<u>Day 1 of gestation</u>	<u>Number of mice</u>	<u>Group</u>
Saturday	64	A
Sunday	64	B
Monday	64	C
Tuesday	48	D
Wednesday	48	E

The groups are treated as follows:

Group A - 32 mice are nontreated controls. Treat 32 mice on gestational day 14; 24 with vitamin A and 8 with corn oil.

Group B - treat 32 mice on gestational day 11; 24 with vitamin A and 8 with corn oil. Treat the remaining 32 mice on gestational day 13; 24 with vitamin A and 8 with corn oil.

Group C - treat 32 mice on gestational day 10; 24 with vitamin A and 8 with corn oil. Treat remaining 32 mice on gestational day 12; 24 with vitamin A and 8 with corn oil.

Group D - treat 48 mice on gestational day 9; 32 with vitamin A and 16 with corn oil.

Group E - treat 48 mice on gestational day 8; 32 with vitamin A and 16 with corn oil.

All treatments occur on either Wednesday or Friday as may be seen in the following schedule:

<u>Group</u>	<u>Day of gestation</u>	<u>No. of mice</u>	<u>Treatment</u>
<u>Wednesday</u>			
B	11	24	vitamin A
B	11	8	corn oil
C	10	24	vitamin A
C	10	8	corn oil
D	9	32	vitamin A
D	9	16	corn oil
E	8	32	vitamin A
E	8	16	corn oil
<u>Friday</u>			
A	14	24	vitamin A
A	14	8	corn oil
B	13	24	vitamin A
B	13	8	corn oil
C	12	24	vitamin A
C	12	8	corn oil

Beginning on the following Monday, the mice are sacrificed on gestational day 17 according to the following schedule. The fetuses are stored in Bouin's solution until necropsied.

<u>Weekday</u>	<u>Group</u>	<u>Day they were treated</u>	<u>No. of mice</u>
Monday	A	nontreated controls + day 14	64
Tuesday	B	day 11 & 13	64
Wednesday	C	day 10 & 12	64
Thursday	D	day 9	48
Friday	E	day 8	48